

**Bayer CropScience**

**Response to the Clothianidin Petition**

Comments to Docket # EPA-HQ-OPP-2012-0334

September 24, 2012

**Technical Reviews of Several Recently Published Scientific Articles concerning Bee Health and the use of Neonicotinoids**

During the first half of 2012, a number of articles have been published in scientific journals that report research undertaken to evaluate potential effects of the neonicotinoid class of pesticides on honey bees and other pollinators. Several of these studies were accompanied by considerable media attention claiming singular importance in explaining overall pollinator health status without full consideration of many years of research on the risks of neonicotinoids to bees.

Bayer CropScience (BCS) scientists have reviewed each of these studies to determine the relevancy, limitations, and any deficiencies of the reported results when conducting a risk assessment for neonicotinoid products. These reviews have previously been submitted to EPA<sup>a</sup>. It should be noted that while all the studies were conducted on neonicotinoids, not all were conducted on clothianidin, making their relevance to the clothianidin petition limited. As the petitioners nevertheless chose to rely predominantly on references relating to pesticides other than clothianidin in their petition, we feel it is germane to include these reviews here as part of a balanced response. The articles BCS reviewed are:

1. Christian H. Krupke et.al., Multiple Routes of Pesticide Exposure for Honey Bees Living near Agricultural Fields, **PloS ONE** vol.7, iss. 1, January 2012;
2. Jeffery S. Pettis et.al., Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*, **Naturwissenschaften** vol. 99: 153- 158, 13 January 2012;
3. Penelope R. Whitehorn, et.al., Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production, **SCIENCE** vol. 336, 20 April 2012;
4. Mickaël Henry, et.al., A Common Pesticide Decreases Foraging Success and Survival in Honey Bees, **SCIENCE** vol. 336, 20 April 2012;

5. Andrea Tapparo et.al. Assessment of the Environmental Exposure of Honeybees to Particulate Matter Containing Neonicotinoid Insecticides Coming from Corn Coated Seeds, **Environ. Sci. Technol.** vol. 46, 2592-2599, 2012;
6. Chensheng Lu et.al. *In situ* replication of honey bee colony collapse disorder, **Bulletin of Insectology** 65 (1), 99-106, 2012.

Not included in the initial review is an assessment of the relevance of the following, more recent study, which has been referenced by the petitioners:

7. Daren M. Eiri and J. C. Nieh. A nicotinic acetylcholine receptor agonist affects honey bee sucrose responsiveness and decreases waggle dancing. **Journal of Experimental Biology** 215, 2022-2029, 2012.

When considering the relevancy of this study to a neonicotinoid risk assessment, it must be considered that exposure took place under artificial conditions in a test system that has not been validated. The test concentrations are field unrealistic, with even the lower one tested being 5-10 times higher than realistic nectar concentrations that might originate from seed treatment applications. Potential effects to the colonies were not investigated, only effects on individuals. The study provides no evidence that real-world exposures would lead to an adverse impact to a colony.

### Reference Cited

- a) Halder, C. A., 2012. Technical Reviews of Several Recently Published Scientific Articles Concerning Bee Health and the Use of Neonicotinoids, Bayer CropScience Report #US0278, May 10, 2012. MRID # 48828701

## **Bayer CropScience Statement on the Findings of the Study:**

### **KRUPKE et al. (2012): Multiple Routes of Pesticide Exposure for Honey Bees Living Near Agricultural Fields *PLoS ONE* **7** (1) 1-8**

#### **Contents of the publication**

The publication of KRUPKE et al. (2012) reports the findings of a study on potential routes of exposures for honeybees to pesticides, especially to Neonicotinoids, conducted in a corn-growing region in USA in 2010. The study was initiated in response to reports of bee kills at Indiana apiaries in spring of 2010 which coincided with the corn planting period in the area and which were believed to be related to neonicotinoid seed treatment products.

In the first part of the study, one half of the study field of a ca. 2.1 ha was sown with corn seeds treated with Clothianidin at a dressing rate of 1.25 mg a.s./kernel with a vacuum-pneumatic drilling machine, the other half with untreated corn seeds. Commercial bee hives were set up within and around the field. Samples for analysis of neonicotinoids were taken from the soils of fields around the study field, from waste talc in the planter that was added to the seeds before drilling, from pollen of treated and untreated corn plants on the study field, and from pollen loads of forager bees returning to the study hives. Additional test plots were drilled using the same planter in 2011 with commercially-obtained corn seeds treated with various rates of Thiamethoxam or Clothianidin and additional samples of the waste talc were collected. Key analytical findings are depicted in the below table:

<b>Matrix</b>	<b>Clothianidin [ppb]</b>	<b>Thiamethoxam [ppb]</b>
Soil of surrounding fields	2.1 - 9.6	n.d.
Talc from the planter	4,900,000 - 15,030,000	68,000 - 13,240,000
Maize pollen from treated plants (study plot)	3.9	1.7
Pollen loads collected before drilling of the study plot	n.d. - 88	n.d. - 7.4
Pollen loads collected after drilling of the study plot	n.d. - 12	n.d.

n.d. = not detected

In the second part of the study, samples were taken from an apiary in Indiana where in 2011 increased bee mortality was reported that was coinciding with the corn planting season. Samples were taken from weeds growing close to corn fields in the surroundings, from dead and from live bees found near the hives of the apiary, as well as from recently stored pollen and nectar taken from the apiary's hives.

Key analytical findings are depicted in the below table:

Matrix	Clothianidin [ppb]	Thiamethoxam [ppb]
Dead bees	3.8-13.3	n.d.
Healthy bees	n.d.	n.d.
Pollen in hive ("sick")	10.7	20.4
Pollen in hive ("healthy")	2.9	6.2
Weeds near study field	1.1-9.4	n.d.-2.9

n.d. = not detected

These findings are claimed to give evidence about different exposure routes for honeybees to neonicotinoids originating from seed treatment products, which are basically exposure to soil dust from previously treated fields that contains neonicotinoid residues, exposure to abraded dust from the seed treatment emitted during the drilling process, and exposure to systemic residues in nectar and pollen of treated crops (or succeeding crops/weeds on treated fields). By these multiple pathways of exposure, bee colonies in areas where neonicotinoid seed treatment is applied would then be exposed throughout the growing season.

#### Comments from the BCS perspective

The publication of KRUPKE et al. (2012) presents some data on potential exposure routes to neonicotinoid seed treatment products; basically none of these are fundamentally new, all have already been suggested and investigated in previous publications or studies (PISTORIUS et al. 2009; NIKOLAKIS et al. 2009; HERBST et al. 2010; MARZARO et al. 2011). The presented figures are largely consistent with the data on those exposure routes that already had been collected in previous studies. However, there are some points in particular related to the interpretation of the data surveyed that require clarification or comment:

**Soil dust as exposure route:** One of the discussed potential routes of exposure is via dust from soils that contain neonicotinoid residues from previous croppings. There may indeed be neonicotinoid residues in soils that were planted with seed-treated crops, but these are very low. The highest Clothianidin residue soil level found in this study was 9.6 ppb. Using EPA's contact LD<sub>50</sub> for Clothianidin of 0.0439 µg/bee, a bee would have to be exposed to 4.6 g of soil dust, which is equivalent to 46 times its body weight. Clearly, soil dust containing residue levels reported by Krupke et al. is not a toxicologically significant exposure route for honeybees.

**Waste talc samples from planters:** It is well known that abraded dust from neonicotinoid seed treatment products can contain intrinsically highly toxic insecticide concentrations, which is consequently also true for talcum added to the treated seeds in the planter. This was confirmed in the study of KRUPKE et al. (2012). However, while their results suggest high intrinsic toxicity of these dusts, they do not allow inferences to be made about the risk potential for bees, as they do not give any information about the exposure levels of bees to dusts emitted by corn planters and deposited on

bee attractive flowers. The measurements they reported were made on the waste material left in the planter after drilling, rather than on the dust emitted from it during drilling. This waste material contained broken off pieces of the seeds and seed coatings in addition to talc (Brian Eitzer, personal communication). Because these samples were of material that “dropped out” of the planter exhaust airstream, they likely contained coarser, heavier particles than the dust emitted by the planters. The insecticide concentration of this coarse material may not be representative of that of the dust emitted from the planter. Of more relevance to the assessment of exposure of honey bees to planter-emitted dust would be samples of dust deposition on flowers from areas immediately adjacent and downwind from the planted field. KRUPKE et al. (2012) did not evaluate this, although they did collect and analyze whole dandelion flowers that were growing near recently planted cornfields during their investigation of a 2011 bee kill event. These samples contained only very low levels of neonicotinoids. While of questionable relevance to assessing bee exposure to abraded seed dust released during planting, the high concentrations in the waste talc reported by KRUPKE et al. (2012) do suggest that farmers should take care not to blow waste material on to blooming crops or weeds when cleaning out planting equipment.

**Systemic residues in maize pollen:** Figures displayed are consistent with previous findings from comprehensive field residue studies (SCHMUCK & KEPPLER 2003, unpublished data). The residue levels are not of concern since they are well below the established colony field no-observed-effect concentration of 20 ppb. However, one point should be highlighted: in the pollen sample from Clothianidin-treated maize, both Clothianidin and Thiamethoxam were found. As Thiamethoxam is degraded to Clothianidin, but not vice versa, this is not possible and suggests that either samples were contaminated or mixed up.

**Residues in pollen loads:** Residues in pollen loads that forager bees carried to the study hives were very variable and inconsistent. At planting and immediately following planting residues were low and below a level of concern. Anomalously, there were some higher residues immediately prior to planting. There was no correlation between the amount of corn pollen, as a percentage of total pollen, in a sample and the residue level of neonicotinoids. Therefore it is clear that the residues in this matrix that were found pre-planting are not related to the seed treatment on the study field, and most likely result from an unknown source of contamination.

**Synergistic effects:** The co-occurrence of certain fungicides and neonicotinoids in bee-collected pollen is pointed out by the authors, and they bring up concerns about potential synergistic effects between both types of compounds under reference to IWASA et al. (2004). It should however be noted that IWASA et al. report about synergistic effect potentials between fungicides and cyano-substituted neonicotinoids (e.g. Acetamiprid, Thiacloprid), and found no significant synergistic effects between fungicides and nitro-substituted neonicotinoids (e.g. Imidacloprid, Thiamethoxam, Clothianidin). These two classes of neonicotinoids behave fundamentally different in this respect. Synergistic effects between fungicides and nitro-substituted neonicotinoids have according to our knowledge never been documented in scientific research.

**Lack of Observable Adverse Effects in the 2010 Field Experiment:** The 2010 field experiment appears to have been designed to simulate conditions thought to be responsible for the bee kill incident at the Purdue apiaries earlier that year. Four honey bee colonies were placed around the perimeter of a test plot that was then planted with seeds with maximum Clothianidin treatment rate, and four additional colonies were placed around an adjacent test plot which was planted with untreated seeds. The paper did not mention whether any adverse effects were observed in these colonies seemingly placed in “harm’s way”. These colonies were visited daily for purposes of collecting samples from pollen traps and it seems reasonable to assume that if significant numbers of dead or dying bees were present, this would have been observed. In a recent phone conversation with BCS scientists, Christian Krupke confirmed that no unusual mortalities or signs of pesticide intoxication were observed at these colonies.

**Investigation of 2011 bee mortalities:** From the analytical results depicted, it appears possible that the surveyed bee colonies had been exposed to a neonicotinoid seed treatment product. Residue levels of Clothianidin found in dead bees are comparable to findings after a Clothianidin incident in Germany in 2008. Findings of Clothianidin and of Thiamethoxam in in-hive matrices and on weeds from the surroundings suggest that Thiamethoxam was the compound they were exposed to. There was no Thiamethoxam found in dead bees, but this may be explained by its relatively fast metabolic conversion to Clothianidin in bees. In-hive samples were taken from two hives of the apiary, one “sick” and one “healthy”. Both terms were defined on the basis of presence or absence of dead bees found in front of the hive, which considering the complexity of a bee colony is a quite insufficient criterion. Residues of Thiamethoxam and Clothianidin were found in both the “healthy” and the “sick” hive”, but at somewhat higher levels in the “sick” colonies. However, as the sample size was very small (just two colonies!) there is no way of doing a scientifically sound correlation between the health status of the colonies and the residue levels found. In summary, the data presented basically demonstrate that bee hives from the sampled apiary were exposed to a neonicotinoid, most probably Thiamethoxam. That individual exposed worker bees have been killed is not unlikely. Nonetheless, no effect to the observed colonies was documented. The available evidence does not identify a route of exposure, as the residue levels in the in-hive pollen and the flowers from adjacent fields are not sufficiently high to result in bee mortality. Coincidence of the mortalities with planting season and spatial proximity with typically seed-treated crops support the plausibility of an assumption that dust from seed treatment was the route of exposure, but information regarding the circumstances and conditions that resulted in the exposure (e.g. seed treatment quality, planter types and equipment, agricultural practice, tillage system) are lacking. Therefore, based on the presented data, it is not possible to fully evaluate the incident, nor to exclude for example the possibility that the incident was caused by inappropriate practices related to handling or sowing treated seeds.

Reports of pesticide bee kill incidents associated with corn planting in the US Midwest have been extremely infrequent, suggesting that this is not a common route of exposure in this type of environment, and is being effectively avoided. In other environments, effective measures have been

developed and implemented to minimize exposure (see e.g. NIKOLAKIS et al. 2009, HERBST et al. 2010, EUROPEAN COMMISSION 2010). If the appropriate measures are correctly applied, exposure should be kept at a minimum such that the intrinsic toxicity of dusts should play a minor role in the evaluation of a potential risk. The KRUPKE et al. (2012) study may have identified another agriculture management practice in need of attention, namely the proper clean up and disposal of waste talc after planting. The two bee kill incidents reported KRUPKE et al. (2012) appeared to be of minor severity, with no indication of any colony deaths. The Purdue University colonies affected in 2010 all reportedly recovered to normal strength within a few weeks (Greg Hunt, personal communication). The 2011 incident appears to have been similar, although details were not given in the paper. While these incidents are regrettable, they in no way suggest that use of neonicotinoid insecticides as a corn seed treatment play any role at all in the high annual colony loss rates or Colony Collapse Disorder syndrome that some US Beekeepers have reported in recent years. Bayer CropScience is unaware of even a single bee colony loss in the United States that can be reasonably attributed to the use of Clothianidin, Imidacloprid or any other neonicotinoid as a corn seed treatment.

**Correction of Technical Errors or Misleading Statements about Risk:** Several statements made in the paper are incorrect or highly misleading. First, it was stated that some of the pollen sample concentrations exceeded the oral LD<sub>50</sub>. There was one pollen sample with a total neonicotinoid concentration of 95.4 ppb (88 ppb clothianidin + 7.4 ppb thiamethoxam). For a nurse bee weighing 100 mg and consuming 6.5 mg of pollen with a concentration of 95.4 ppb would result in a dose of 0.62 ng, which is far below the LD<sub>50</sub>. Consumption of the pollen with the average concentration reported for bee-collected pollen would result in daily ingestion of only 2% of the LD<sub>50</sub>.

Second, a related statement made in the paper was “A bee will consume 65 mg of pollen during the 10 day period it spends as a nurse bee, therefore a concentration of 20 ng/g (ppb) in pollen would correspond to a dose of 1.3 ng (65 mg x 20 ng/g) or almost 50% of the oral LD<sub>50</sub> of ca. 2.8 ng/bee.” This statement is accurate, but it is highly misleading to compare the dose ingested over 10 days to an acute oral LD<sub>50</sub>. Bees rapidly metabolize and excrete neonicotinoids so that non-toxic concentrations do not build up over multiple days to become toxic concentrations. The correct approach is to compare the dose ingested per day with the LD<sub>50</sub> (and even this will overestimate the real risk). In this case, the daily dose 0.13 ng/bee/d represents <5% of the LD<sub>50</sub>. A chronic (10-day) feeding study with young adult honey bees established a no-observed-effect level of 0.38 ng/bee/d for clothianidin. Thus, the scenario Krupke et al. hypothesized clearly poses a minimal risk.

Finally, it was stated that pollen contaminated with levels of neonicotinoids similar to those shown in our results has been known to impair pollinator health. Three citations were given in support. The first is a study that evaluated changes in the frequency of the proboscis extension reflex of individual honey bees in a highly artificial laboratory assay. The relevance of changes in this endpoint to the health of bee colonies in the field has not been demonstrated. The second citation is a review article that cites the first study and presents no original data. The third citation refers to sublethal effects related to the foraging activity of bumblebees in laboratory or greenhouse study setups. The data

presented are certainly of scientific interest, but it is not possible to deduce from them any general statement about an “impairment of pollinator health”.

**Conclusion:** In summary, it can be stated that the study of KRUPKE et al. (2012) does not provide any fundamentally new evidence about honeybee exposure to neonicotinoid seed treatment products. The exposure levels reported in soil, pollen and nectar are generally consistent with previous research and were not high enough to represent a significant risk for honey bees. Higher concentrations found in waste talc collected from inside pneumatic equipment post-planting represent an intrinsic hazard to honey bees, however actual exposure of bees to this material was not demonstrated and would appear to be preventable. The paper reports some information documenting the occurrence of two minor bee kill incidents that were coincident with planting of treated corn seeds that may have been caused by neonicotinoid exposure. However, the available information is insufficient to determine the route of exposure. In their field experiment, low exposure levels and no adverse effects were observed for bee colonies placed “in harm’s way” around the perimeter of a field as it was planted with treated corn seeds. Overall, the publication represents an interesting case study, but it does not provide any significant new insights into exposure of honeybees to neonicotinoid insecticides.

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Christian MAUS, David FISCHER, Iain KELLY & Dick ROGERS

Bayer CropScience LP  
2 T.W. Alexander Drive  
Research Triangle Park, NC 27709  
February 21, 2012

**Statement on the findings of the study:**

**PETTIS et al. (2012): Pesticide Exposure in Honey Bees Results in Increased Levels  
of the Gut Pathogen *Nosema***

**Naturwissenschaften, DOI 10.1007/s00114-011-0881-1**

**Contents of the publication**

In the recently issued publication of PETTIS et al. (2012), interactions between chronic, sublethal exposure of honeybee colonies to Imidacloprid, and the infection of individual honeybees with the fungal gut parasite *Nosema* are investigated.

Three treatment groups were set up in the study, each consisting of ten bee hives. One group was fed with artificial diet containing 20 ppb Imidacloprid, the second with 5 ppb Imidacloprid, and the third remained untreated as control group. Exposure time was 10 weeks. After 5 weeks, brood combs were removed, and newly emerged young bees were infested with *Nosema* spores (ca. 330,000 spores per bee). Mortality of the infested bees was recorded, and after 12 days, *Nosema* spore level was determined for each individual bee. In a second trial, bees that emerged from combs taken out of exposed colonies after 8 weeks were infested with two different doses of *Nosema* spores (ca. 330,000 and 33,000 per bee) in the same experimental setup.

In none of the experiments, an increased mortality rate was observed in any treatment group, mortality always remained below 20%. In the first trial, bees from colonies chronically exposed to Imidacloprid had higher *Nosema* spore counts (both treatment groups ca. 700,000 spores/bee) than bees from the control group (ca. 200,000 spores/bee). In the second trial, initial dosage of bees with spores had no effect on the eventual infestation level, but bees from control colonies had lower levels than bees from Imidacloprid-exposed colonies. However, there was no dose-response relationship in spore counts, and differences between treatment and control were less pronounced than in the first trial (control: ca. 1,000,000 spores/bee; 5 ppb: ca. 1,750,000 spores/bee; 20 ppb: ca. 1,500,000 spores/bee). At the end of the trials, eight of the thirty experimental colonies were found infested with *Nosema* (by means of natural infection), but there was no correlation between frequency and severity of infestation, and Imidacloprid exposure: three control colonies (avg. spore counts 4,300,000 spores/bee), three colonies of the 5 ppb group (avg. spore count 2,900,000 spores/bee), and two colonies of the 20 ppb group (avg. spore count 500,000 spores/bee) showed *Nosema* infestations.

From the reported findings, the authors conclude an interaction between sub-lethal exposure to Imidacloprid at the colony level and *Nosema* spore production in individual bees, and postulate that sublethal exposure of honeybee colonies to pesticides may cause adverse effects by making them more susceptible to pathogens, that have so far been overlooked in the pesticide risk assessment.

### Comments from the BCS perspective

The study of PETTIS et al. (2012) appears technically well conducted and well reported. It is common sense that multiple stressors may interact with each other, and that an organism exposed to a certain stressor may be more susceptible of the effects of other stressors. In so far, the reported results as such do not appear implausible. Nevertheless, there are some aspects to consider when drawing conclusions from the results of the study.

- Although the exposure to Imidacloprid took place under field conditions with entire bee colonies, the *Nosema* infestation and exposure part of the study was conducted under artificial laboratory conditions with individual worker bees out of the context of the colony and its complex interactions and compensation mechanisms. In such an artificial laboratory environment, bees may react quite different from a situation under realistic field conditions. There were already many cases where a sublethal stressor was shown to induce certain effects in the laboratory, which could then, however, not be recovered under field conditions, or where sublethal effects that were seen in the laboratory proved not to be biologically relevant under realistic conditions. Therefore, the results of the study referred to here do not provide a proof of an adverse effect to bee colonies by the exposure to sublethal doses of a neonicotinoid (although this is implied in the paper), but at the utmost indicates an intrinsic effect potential. There is no evidence that this could cause a hazard to bee colonies under realistic conditions.
- This is further supported by the observations of PETTIS et al. (2012) regarding the study colonies that remained in the field and were the source for the combs for emergence of the bees for the laboratory part of the study. There was not only no positive correlation between Imidacloprid exposure and natural *Nosema* infection in terms of number of infested colonies, but the infested control colonies had by far the highest infestation levels, followed by the colonies exposed to low Imidacloprid concentrations, and the lowest spore counts were recorded in the colonies exposed to the highest pesticide concentration. This once more underlines the fact that field conditions are fundamentally different from what we can test in the laboratory, and that laboratory data can not be 1 : 1 extrapolated to the field.
- The endpoints measured in the reported study, like *Nosema* infestation are complex and influenced by plenty of partly unknown factors; no standards have been defined so far, and little is known about the intrinsic variability of the endpoints. This is for instance mirrored by the fact that in the first experiment the infestation with 330,000 spores/bee consistently resulted in a spore level of 700,000 spores/bee, whereas in the second trial the infestation with just 1/10<sup>th</sup> of the spores lead to final spore levels of more than 2,000,000/bee, with similar levels in bees originally infested with 330,000 spores/bee. This complete lack of a dose-response relationship underlines the high intrinsic variability of the endpoint and makes it extremely difficult to distinguish true correlations from erratic findings.

- Before concluding on any consistent and relevant synergistic effect, the reproducibility of the reported findings has to be demonstrated. Findings of a higher sensitivity of honeybees exposed to sublethal concentrations of Imidacloprid to *Nosema* infestation has also been reported in another laboratory study (ALAUX et al. 2009), but a third study from the Bee Institute of Celle/Germany, did not find interactions between sublethal exposure to Imidacloprid and a challenge with *Nosema* infestation (WEHLING et al. 2006, 2009). Likewise, the observation of PETTIS et al. (2012) that individual bees from the treatment groups exposed to Imidacloprid were more severely infested with *Nosema* than the control groups are in contradiction with the conclusion of ALAUX et al. (2009) that Imidacloprid may have a slight suppressing effect to *Nosema* (yet this seems to be confirmed again by the field colony data from PETTIS et al. 2012). These inconsistencies clearly point at the fact that for testing of sublethal effects and of multiple stressor effects to honeybees there are no fully technically mature and validated test designs available so that it is not surprising that study results may be inconsistent and not reproducible.
- Another point that should be emphasized is the fact that in none of the treatment groups there was any increased mortality observed. This demonstrates that vitality of bees originating from colonies chronically exposed to Imidacloprid at levels up to 20 ppb is not negatively affected, even not under aggravated conditions like an artificial infestation with *Nosema*. This confirms the established chronic field NOAEC for Imidacloprid at 20 ppb.
- Finally, the hypothesis that sublethal exposure to pesticides makes bees more susceptible to diseases or parasites under field condition is contradicted by the findings of several field monitoring projects where the occurrence of pathogens is surveyed as well as the exposure to pesticides under realistic field conditions (e.g. VAN ENGELSDORP et al. 2009, CHAUZAT et al. 2010a, b, GENERSCH et al. 2010, HIGES et al. 2010). None of the mentioned studies makes mention of any correlation between infestation with certain pathogens and the exposure to pesticide residues, or between findings of pesticide residues and increased colony mortality, as it would have to be expected if the hypothesis would be true. The fact that no correlation between pesticide exposure and honeybee colony mortality is found does obviously not support the hypothesis of PETTIS et al. (2012) that interactions between pesticides and pathogens might be a major contributor to increased mortality of honey bee colonies worldwide, and their concern that there might in terms of this be major gaps in the existing testing and risk assessment procedures for pesticides and their effects to honey bees.

In summary, it can be stated that the study of PETTIS et al. (2012) does not convincingly substantiate the assumption of any adverse effects of the exposure of honeybees to sublethal doses of Imidacloprid in combination with other stressors like diseases or pathogens under realistic exposure conditions in the field.

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Dr. Christian MAUS, 2012-01-27

**Neonicotinoids pesticide reduces bumblebee colony growth and queen production**

Penelope R. WHITEHORN<sup>1</sup>, Stephanie O'CONNOR<sup>1</sup>, Felix L. WACKERS<sup>2</sup> & Dave GOULSON<sup>1</sup>.

<sup>1</sup>*School Natural Sciences, University of Stirling, Stirling, FK9 4LA, UK.*

<sup>2</sup>*Lancaster University, LEC, Lancaster, LA1 4YQ, UK*

This is an interesting study that evaluated effects on bumble bee colony development from exposure to Imidacloprid-contaminated diet. Replicate colonies of *Bombus terrestris* were fed with pollen and nectar for two weeks under laboratory conditions. The colonies received *ad libitum* pollen and sugar water; in the control group, sugar water and pollen were uncontaminated, in the treatment groups, sugar water and pollen were spiked with 6 and 0.7 ppb or with 12 and 1.4 ppb Imidacloprid, respectively. After two weeks of exposure in the laboratory, colonies were placed at a field location remote from intensive pesticide use to forage freely and consume natural pollen and nectar for a period of 6 additional weeks. The weight of the colonies was assessed each week and the number of individual drones, workers, new queens, pupae and empty cells was assessed at the termination of the study. All the colonies of all treatment groups developed and produced queens; weight development was comparable between treatment and control during the exposure time (first two weeks). From week 3 onwards, however, the control colonies gained more cumulative weight and produced significantly more queens than the treatment colonies. Queen production in bumble bee colonies can depend on colony size, with larger colonies producing more queens than small colonies, so the authors conclude that the exposed colonies grew less than the control colonies due to effects of Imidacloprid sublethal exposure, and therefore produced fewer queens.

**Comments from the BCS Perspective:**

1. The test design applied by WHITEHORN et al. (2012) is not validated; therefore it is not clear whether the results presented would be reproducible at all. Experience with ecotoxicological study designs for bees has shown that study validation is essential to assure generating scientifically sound results.
2. The reported lower colony weight of treatment colonies compared to control colonies is basically just a difference between different experimental groups; although there may be a statistically significant difference in this endpoint, there is no evidence at all that this represents an **adverse** effect, or, respectively, a **biologically significant** effect. In honey bees, for instance, hive weight gain is predominantly determined by the quantity of nectar stores in the hive, which can be very different between individual colonies even when set up at the same location under the same environmental conditions, without indicating differences in colony vitality. Although bumblebees do not have as extensive nectar stores as honeybees (as the authors note), *B. terrestris* does

indeed store nectar (see e.g. DORNHAUS & CHITTKA 2004, 2005), moreover a nest consists of many other components (nesting material, pollen stores etc.) that influence its weight, so that the colony weight parameter would not be very significant to deduce an adverse effect to the organisms of the colony.

3. The number of workers per colony (i.e. the colony strength) at study termination was not significantly different among the different treatment groups. Therefore the argument of the authors that “even a small drop in colony size may bring [the colony] below the threshold for queen production” is not very logical, as colony size is defined by number of individuals in the colony and not by nest weight. In any case, there are no data available in the scientific literature that show that queen production of a bumblebee colony is dependent on nest weight.
4. The differences in weight gain of colonies can have several causes, and this parameter is highly variable. In a previous publication (GOULSON et al. 2002), one of the authors shows that changes in weight of bumblebee nests situated in three different habitat types differs significantly depending on their location and the associated available resources, but can also be influenced by the presence of a parasite (*Aphonimia socialia*) in the colonies with lower weight gain. In the study of WHITEHORN et al. (2012), no exact description of such alternative parameters that might likewise influence colony weight development. One particularity that shows how variable this parameter can be is the comparison of the values of net cumulative weight gain between the colonies used in the previous study (GOULSON et al. 2002) and the current study: the hives that had the weakest development gained around 400 g within 4 weeks in the older publication, whereas in the current study the colonies that developed most strongly gained only 200 g within the same time span. This variability in the endpoint of weight gain highlights again that the test design is not established and validated, and drawing conclusions on the development of bumblebee colonies under the influence of sublethal exposure concentrations of Neonicotinoids on the basis of the findings presented here is very problematic.
5. How many new queens are produced by a bumblebee colony in one year's cycle is a parameter that is so far unpredictable and governed by processes and factors that are poorly understood (DUCHATEAU et al. 2004, GOULSON 2006). Although the size of the colony, normally expressed as the number of workers, may have an impact on when the production of queens is triggered in a colony, it is not the only factor. For example, a study comparing the production of new queens and drones between several colonies reared at different times shows that one of the main factors affecting the production on new queens is the hibernation conditions of the founder queen (DUCHATEAU et al. 2004). In the study under discussion here, there is no mention of these factors, and it is not clear at which point in the development cycle of the colonies the exposure took place. Therefore it is quite possible that factors other than pesticide exposure, that were not assessed and not taken into consideration, influenced the outcome of the study regarding the queen production endpoint.
6. The authors indicate that they chose the low test concentrations (6 ppb in pollen and 0.7 ppb in nectar) because this is the level found in oil-seed rape, however the reference they cited in support (BONMATIN et al. 2003) did not include any original research measurements of residue levels in oil-seed rape nectar and pollen, but instead reported the levels of 4.4 to 7.6 ppb in pollen

and 0.6 to 0.8 ppb in nectar that were determined in an unpublished study by SCOTT-DUPREE & SPIVAK (2001). These data, which came from just two experimental test plots, are not broadly representative of what bees encounter under conditions of commercial use. They represent high end, rather than typical field concentrations. Residues of Imidacloprid in nectar and pollen of seed-treated crops like sunflower or oilseed rape are in most cases significantly lower, as outlined in SCHMUCK et al. (2004) and MAUS et al. (2003) where a much higher number of residue data points were evaluated: "In sunflower crops, the Imidacloprid residue levels in nectar and pollen were in all but one sample below the LOD of 1.5 ppb. Only one out of 18 analysed samples showed a higher residue level of 1.6 ppb. In rape, residue levels between < 1.5 and 5 ppb were recorded in the nectar and pollen samples. Only in one out of 15 analysed samples, a higher residue level was detected (7.8 ppb in a pollen sample)."

7. Furthermore, a continuous exposure to a contaminated diet over two weeks in a no-choice scenario in the laboratory will in any case lead to exaggerated exposure conditions that are not comparable to a situation as prevailing in the field. Under realistic conditions, a bumblebee colony, even when exposed to a highly attractive, seed-treated crop, would not source its complete nectar and pollen supply exclusively from this culture over two full weeks. Additional food sources would be exploited as well (especially by bumblebees which only have a weakly pronounced individual fixation or preference for certain types of flowers, and which do not communicate with their nestmates to attract them to a certain type of flower). This would lead to a significant dilution of residues in the colony's food stores. This is even true for a scenario as outlined by WHITEHORN et al. (2012) where in a 10 x 20 km patch of land 100% of the area is in a 1 km radius of oilseed rape fields.
8. The authors indicate that no field studies have examined of the impact of Imidacloprid soil-systemic treatments to bumble bee colonies in the field. This is incorrect. TASEI et al. (2001) and GELS & POTTER (2002) reported no effects on bumble bees for use of Imidacloprid as a sunflower seed treatment and application to lawns containing clover, respectively, when the compound was applied according to label directions. Foliar deposits applications not followed by irrigation (a violation of the product label) were in contrast found to be hazardous to bumblebees by GELS & POTTER (2002).
9. The authors suggest that based on their results the use of neonicotinoids may be having considerable negative impact on wild bumblebee populations across the developed world. This is a highly speculative statement. The authors do not adequately discuss the uncertainties inherent in extrapolation of their study results, a main one being whether the exposure levels they tested are representative of what bumble bees experience in real field scenarios. A critical assumption they are making is that nectar concentrations that bumble bees are exposed to in the real world are 6 ppb or greater since they did not test any lower concentrations. This assumption is based on limited field data from one small-plot field study that did even include bumble bees. Other field studies have found clearly lower levels of neonicotinoids in nectar and pollen of seed-treated crops when evaluating a more extended pool of data (e.g. MAUS et al. 2003, SCHMUCK et al. 2004, CUTLER & SCOTT-DUPREE, 2007).

10. Finally, there are several statements in the paper that are simply untrue or incorrect in the given context. These include:

- In the context of reviewing past bee studies of neonicotinoids, the authors state is “unclear what impact this exposure [to Neonicotinoids] has on bee colonies under field conditions”. In reality, it is quite clear that effects are not observed in the field. There are a plenty of field studies available that investigate exactly this question and conclude no adverse effects by exposure under realistic conditions (e.g. SCHMUCK & KEPPLER 2003, MAUS et al. 2003, SCHMUCK et al. 2004, CUTLER & SCOTT-DUPREE, 2007, NGUYEN et al. 2009, and many more). That all these field data were largely ignored shows that the authors cited quite selectively from the available literature.
- The reference to 140 different crops treated with Imidacloprid suggests that the authors apparently mix up seed treatment and spray treatments; the majority of these crops are treated with spray formulations. Any spray application of Imidacloprid is prescribed to be conducted out of the flowering period of the crop, so that an exposure of bees and bumblebees can be a-priori be excluded when the application is done correctly.
- The mention of pollen residue levels of Neonicotinoids (here: Clothianidin) up to 88 µg/kg refers to a recent publication of KRUPKE et al. (2012). This unique finding from one sample is an extreme outlier in comparison to the many hundreds of pollen samples that have been reported for the same compound in other studies (e.g. SCHMUCK & KEPPLER 2003, LIEBIG et al. 2008 and BCS unpublished data). Therefore, the origin of the high residue level in the aforementioned sample must be considered unclear and cannot necessarily be attributed to systemic residues from seed treatment.

## Conclusions

In conclusion it can be stated that the results presented by WHITEHORN et al. (2012) originate from a study conducted under artificial conditions that may have resulted in higher exposures to Imidacloprid than are likely to occur at real crop fields, and that the observed effects, besides being of questionable reproducibility, may not necessarily be attributable to exposure to Imidacloprid. The conclusions drawn by the authors are both speculative and at odds with the results of previous studies. The overall weight of the currently available evidence does not support the authors' conclusion that exposure to Neonicotinoids under realistic field conditions may be causing widespread harm to bumblebee populations.

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Dr. Maria Teresa ALMANZA, Dr. David L. FISCHER, Dr. Christian MAUS, 2012-04-01

## Notes on Study of HENRY et al. (2012)

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**Title:** A Common Pesticide Decreases Foraging Success and Survival in Honey Bees

**Authors:** Mickaël HENRY<sup>1\*</sup>, Maxime BEGUIN<sup>2</sup>, Fabrice REQUIER<sup>3,4</sup>, Orianne ROLLIN<sup>1,5</sup>, Jean- François ODOUX<sup>4</sup>, Pierrick AUPINEL<sup>4</sup>, Jean APTEL<sup>1</sup>, Sylvie TCHAMITCHIAN<sup>1</sup>, Axel DECOURTYE<sup>5</sup>

<sup>1</sup>INRA, UR406 Abeilles et Environnement, F-84914 Avignon, France.

<sup>2</sup>Association pour le développement de l'apiculture provençale (ADAPI), F-13626 Aix-en-Provence, France.

<sup>3</sup>Centre d'Etudes Biologiques de Chizé, CNRS, UPR1934, F-79360 Beauvoir-sur-Niort, France.

<sup>4</sup>INRA, UE1255, UE Entomologie, F-17700 Surgères, France.

<sup>5</sup>ACTA, UMT PrADE, UR 406 Abeilles et Environnement, F-84914 Avignon, France.

This is an interesting study which employed the use of radio frequency identification (RFID) tags on honey bees to study whether exposure to the Neonicotinoid insecticide Thiamethoxam (manufactured by Syngenta) impairs the ability of forager bees to return to the hive and if so, whether this is likely to have consequences for the long-term survival of the colony. The study is presented in the context of colony collapse disorder, a phenomenon in which adult honey bees rapidly disappear from their colony leaving behind apparently healthy queen and abundant brood and food stores, and other types of colony losses as currently observed in many regions of the world. Some have hypothesized that the disappearance of the adult bees and eventual collapse of colonies may be a

result of sublethal toxicity of Neonicotinoid pesticides since various laboratory assays are claimed to show these compounds impair memory and learning of honey bees at sublethal levels. HENRY et al. (2012) evaluated whether acute dietary exposure to a non-lethal dose of Thiamethoxam causes homing failure (inability of foraging bees to return to their hive) and indirectly whether this could lead to colony failure (hive depopulation). Homing failure rates were determined by direct experimentation. Pollen forager bees were captured as they returned to their hive, held for several hours and after a period of feeding and fasting administered a dose of 1.34 ng Thiamethoxam in 20  $\mu$ L of 50% sugar solution containing 0.067 ng Thiamethoxam/ $\mu$ L. The bees were then released 1 km away from the hive. Test subjects were marked with radio frequency identification tags that permitted automated recording of when these bees returned to the hive. Bees not returning to the hive were assumed to have died and the mortality from homing failure and background causes (determined in control bees) were then inputted into a population model to predict the long term consequences of the pesticide-induced homing failure on the hive colony dynamics.

### **Comments from the BCS Perspective**

1. Actually, the study setup described by the authors is just measuring homing behavior, and not survival or mortality by homing failure. Worker bee mortality and population dynamics in a colony is a very complex natural process which is influenced by many factors, and the natural turnover (i.e. mortality) rate of a full-sized colony is up to 2,000 bees per day (IMDORF et al. 1996). Worker bees that die in terms of this turnover tend to leave their hive before by instinct as a behavioral pattern related to colony hygiene. Therefore, it is an unjustified interpretation to *a-priori* equate differences in numbers of worker bees returning (which does not necessarily constitute an adverse effect on colony level) to a colony with “mortality by homing failure”.

2. The dose level tested was described as being “commonly encountered” by foraging bees in real field situations, but justification for this is not well explained and appears to be based on faulty reasoning. The concentration of the dosing solution was 67 µg/L, which is about 56 ppb when one converts it to a wt/wt measurement (50% sucrose solution has a density of roughly 1.2 mg/µL). This concentration is much greater than the 1-5 ppb levels that have typically been reported in nectar produced by plants grown from Neonicotinoid-treated seeds; even maximum residue levels of Neonicotinoids found in nectar or pollen of seed-treated crops are by far lower than the concentration applied here. HENRY et al. (2012) apparently set their dosing concentrations to produce the maximum cumulative dose bees may ingest over an entire day of feeding, and then administered this dose at once in a single meal. For a Neonicotinoid, which is rapidly metabolized by honey bees, the dose bees can tolerate over an entire day of feeding is much greater than what they can tolerate if ingested as a single meal. An analogous situation would occur if instead of taking 2 pills of a medication every 4 hours for a total of 12 per 24 hours, as is directed by the product label, a person were to take 12 pills at one time. The single dose ingested would be 6 times greater than the maximum recommended. HENRY et al. (2012) have done essentially the same thing. The single dose they administered was >20 times the single dose forager bees obtain when they feed on nectar of flowers of crops grown from Thiamethoxam treated seeds: A report by the French authorities (ANSES 2010) estimated the concentration of Thiamethoxam present in nectar of oil-seed rape flowers to be slightly less than 2 ppb. Nectar forager bees have a limit of about 30 mg they ingest in a single foraging bout. Even if one assumes all of this nectar was consumed as food (rather than brought back to the hive for purposes of making honey), the dose the bee would ingest from 30 mg of nectar with a concentration of 2 ppb (=ng/g) is 0.06 ng. This therefore would appear to represent a worst-case estimate of the acute oral dose that is field relevant. HENRY et al. (2012) gave their bees a dose of 1.34 ng, which is 22 times greater than 0.06 ng. It should likewise be emphasized

that an acute dose of 1.34 ng Thiamethoxam/bee as administered here may already have caused a certain level of mortality as it is relatively close to LD<sub>50</sub> figures established for some Neonicotinoids. It may have been a sublethal dose level, but it was still a very high dose level. It is improper to claim the exposure scenario as described by HENRY et al. (2012) is representative of what bees commonly encounter at real world agricultural sites.

3. Rather than try to calculate the worst-case acute oral dose level bees could experience in the field, as was done above, HENRY et al. (2012) used the data of RORTAIS et al. (2007) to estimate the possible cumulative daily dose for a forager bee. In other words, they sought to give as a single dose the total amount of toxicant taken in over an entire day of feeding. Many substances are toxic when an entire daily intake is administered as a single dose. Even water may be fatal if a person is forced to drink too much at once. Referring back to the medication example, would anyone not expect side effects if a person took 12 pills at once, instead of 2 every 4 hours? HENRY et al. (2012) did not explain the details of how they came up with a target dose level of 1 ng/bee. But clearly, this is not a field relevant exposure level. And the fact that their dosing solution turned out upon chemical analysis to be 134% of nominal is also concerning. This is not “reasonably close” to the nominal amount—usually regulatory authorities consider 80-120% of nominal to be “reasonable”. The reported level (134%) is clearly outside the norm of typical measurement error in preparation of dose levels.
4. The test design applied by HENRY et al. (2012) is not validated; therefore it is not clear whether the results presented would be reproducible at all. Experience with honeybee study designs has shown that study validation is essential to assure generating scientifically sound results. Likewise, the population model applied seems not to be validated, at least not for honeybee population dynamics. Population development of a bee colony is a highly complex thing and we are far away from having fully understood its mechanisms and interdependencies. For instance, recent research results suggest that the individual lifespan of worker bees may be influenced by the

abundance of brood in the colony (e.g. LIEBIG et al. 2012). Such effects that might effectively compensate losses of forager bees are not considered in the applied population model.

5. A few months ago, the study of SCHNEIDER et al. (2012) was published that had a similar purpose and design as the experiments described by HENRY et al. (2012). Using RFID technology, foraging and homing behavior of honeybees exposed to sublethal levels of Imidacloprid and Clothianidin was tested. In contrast to HENRY et al. (2012), SCHNEIDER et al. (2012) tested a set of different exposure concentrations of their test substances (0.15 to 6 ng/bee Imidacloprid and 0.05 to 2 ng/bee Clothianidin), among them concentrations that really may correspond to field-relevant exposure scenarios. They concluded that “at field-relevant doses for nectar and pollen no adverse effect was observed for either substance”. It is highly likely that HENRY et al. (2012) would have come to the same conclusion for their test substance Thiamethoxam, if they would have followed Schneider et al.’s scientifically sound approach to test a range of concentrations that also include field-relevant dose rates. Interestingly, HENRY et al. (2012) mention the work of SCHNEIDER et al. (2012) in their reference list, but do not refer to it in more detail in their discussion, as would be expected given that both studies deal with the same methods and with compounds of the same class of chemicals.
6. In the study of HENRY et al. (2012), there were, as in most studies so far conducted on sublethal effects, only individual bees tested out of the context of the colony. It has been frequently seen that worker bees when sub-lethally exposed to a pesticide react completely differently compared to when a whole colony is exposed to the same compound, or that sublethal effects that were seen in the laboratory on individual bees could not be recovered under field conditions in bee colonies exposed to the respective concentrations of the compound; this has for instance been shown for the proboscis extension reflex test (MAUS et al. 2003; THOMPSON & MAUS 2007; other example in PETTIS et al. 2012). Therefore, an extrapolation from findings on individual

bees exposed under artificial designs to colony effects under field conditions is generally difficult to make, if not even impossible.

7. If exposure of honeybee colonies to sublethal levels of Neonicotinoids under field conditions would in fact lead to a loss of forager bees and eventually a depopulation of the hives, as the authors hypothesize, this should also become evident in field studies where hives are exposed to treated crops. However, more than 30 field studies have been conducted with Neonicotinoids and this kind of effect has never been observed (see for instance MAUS et al. 2003, SCHMUCK et al. 2005, SCHMUCK & KEPPLER 2003).
8. There is no field evidence linking hive depopulations to sublethal exposures to Neonicotinoids. On the contrary, CCD occurrence and other colony losses that have been observed at large scales are not correlated with exposure of honey bee colonies to Neonicotinoids (VAN ENGELSDORP et al. 2009, DELAPLANE 2012) or to exposure of colonies to Neonicotinoid-treated crops (e.g. OTTEN 2003a, b, CHARRIÈRE & NEUMANN 2010). Linkage of Neonicotinoid exposure to declining bee colony health and elevated colony losses has NOT been found in any of the recent regional multifactorial studies of declining bee health (VAN ENGELSDORP et al. 2009, 2010, ROGERS & KEMP 2004, NGUYEN et al. 2009, CHAUZAT et al. 2009, GENERSCH et al. 2010). Finally, in recent scientific reviews of the evidence for whether Neonicotinoid pesticides plays a causal role in bee declines (BLACQUIERE et al. 2012, CRESSWELL et al. 2012), the conclusion reached is there is no evidence that they do.
9. There are several statements in the paper that are simply untrue or incorrect in the given context. These include:
  - a. CCD is not a common phenomenon of the Northern hemisphere as initially stated in the article, but specific to USA; in Europe, for instance, colony losses with CCD symptoms are exceptional (see for instance HENDRIKX et al. 2009, VAN ENGELSDORP et al. 2009, VAN ENGELSDORP & MEIXNER 2010). Even in the USA, many professional apiarists have never seen a single case of CCD. The vast majority of colony losses in the US are not from CCD.

- b. Neonicotinoids are not “especially liable to provoke [...] behavioral trouble.” There have indeed been several studies in which sublethal doses of Neonicotinoids were tested on bees, and behavioral differences were found between treated and untreated individuals. However, in no case it has been shown that these differences indeed would constitute **adverse** effects and would thus have to be classified as “behavioral troubles”.

**Conclusion:** The study demonstrates that sublethal, but nevertheless very high and non-field relevant exposure levels of Thiamethoxam have an effect on homing behavior of forager bees. It does not provide significant new insights into risk assessments for Neonicotinoids. The results are NOT evidence that Neonicotinoids are involved in CCD or otherwise elevated colony losses.

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Dr. David L. FISCHER & Dr. Christian MAUS, 2012-04-01

### **Statement on the findings of the study:**

**TAPPARO et al. (2012):** Assessment of the Environmental Exposure of Honeybees to Particulate Matter Containing Neonicotinoid Insecticides Coming from Corn Coated Seeds

**Authors:** A. TAPPARO, D. MARTON, C. GIORIO, A. ZANELLA, L. SOLDA, M. MARZARO, L. VIVAN, & V. GIROLAMI.

University of Padova, Padova, Italy

Environmental Science and Technology, DOI: 10.1021/es2035152

### **Contents of the publication**

TAPPARO et al. (2012) report on results of field experiments that measured emissions of particulate matter containing neonicotinoid insecticides from the sowing of dressed maize seeds and resulting potential exposure levels for honey bees. Various types of treated corn seeds were sown into a test field using two different types of pneumatic planters and the amount of total particulate matter and active ingredient emitted into the air and deposited at various distances away from the planter or downwind edge of the field were determined. The experiments were run with and without downward deflectors mounted on planter's air exhaust outlet. Two different types of experiments were performed: "mobile sowing" and "static sowing". In the mobile sowing experiments, the planters moved across the field as in normal maize sowing practice. In static sowing experiments, the planter remained in a fixed place but processed seeds as would normally occur during mobile planting. The seeds tested were commercial products of Pioneer Hi-bred that were marketed in 2008, 2009 and 2010. All of these seed batches had Heubach dustmeter measures of <3 g per 100 kg seed.

Insecticide concentrations in samples of exhausted air at the outlet of the air fan ranged from 3.39 to 12.0 mg/m<sup>3</sup> and the mass of insecticide a.i. released per ha planted ranged from 0.43 to 1.53 g. The percentage of the insecticide a.i. present on the seeds that was emitted in the planter air exhaust ranged from 0.52 to 1.85%. Some differences were noted between different batches of seeds, for example between Poncho 1250 seeds from 2009 and 2010. However, the authors indicate that all seed samples emitted significant quantities of insecticide-laden particulate matter.

As part of the static sowing experiments, sugar syrup feeders and honey bee hives were placed so that bees would fly directly through the air exhaust of the planter, and become "powdered" with any emitted dust. After the "static planter" had been allowed to operate for 1 hour, individual bees that had flown across the field were captured and subject to chemical analysis to determine the mass of insecticide on their body. Likewise caged bees were placed for 30 s at distances of 1.00, 2.25, 4.50, 6.75 and 9.00

meters from the airflow emitted by the planter and then analyzed for insecticide load. The concentrations measured in these individual bee samples were highly variable. The bees flying across the field had insecticide loads ranging from 78 to >1000 ng/bee, and the caged bees had similarly high residue loads, with a tendency for the exposure level to decrease with distance away from the air outlet. For comparison, the authors cite the contact LD<sub>50</sub> values for imidacloprid, clothianidin and thiamethoxam of 18, 22 and 30 ng/bee that were reported by IWASA et al. (2004) and suggest their measured residue levels were well above the lethal level. The authors do not mention that these values are for bees dosed with a liquid droplet of the a.i. using acetone as a carrier, and that penetration across the insect cuticle and the resulting LD<sub>50</sub> may differ greatly for a chemical applied as a dust.

Use of the deflectors greatly reduced the mass of particulate matter and insecticide sampled downwind from the planter, but did not have a dramatic effect on the contact dose to individual bees that flew across the field or were caged near the planter in the static sowing experiments.

The authors conclude that particulate matter released by drilling machines during sowing of maize seeds coated with neonicotinoid insecticides represents a significant mechanism of environmental diffusion of these insecticides. Bees flying over the sowing field and approaching the emission cloud of the drilling machine can efficiently intercept the suspended particles being directly contaminated with an elevated dose of insecticide, significantly higher than contact LD<sub>50</sub> values. These exposures therefore represent a concern for both apiculture and crop production based on bee pollination.

### **Comments from the BCS perspective**

The publication of TAPPARO et al. (2012) is one out of several articles published by this group of authors and to fully appreciate the significance of their data, it is necessary to also read their other papers (e.g., MARZARO et al. 2009, GIROLAMI et al. 2012). In prior studies, the authors investigated the possibility that honey bees get into contact with lethal doses of neonicotinoid insecticides when they visit flowering plants where deposition of abraded seed dust has occurred or by ingestion of guttation or dew droplets of the same flowering plants. However, they found the concentrations and bee doses resulting from foraging on such plants for pollen and nectar of flowers or for dew drops and guttation fluids were not high enough to cause mortality. Drift of abraded seed dust to adjacent blooming plants could not explain the bee kill events observed in Italy and which seemed to be associated with the planting of neonicotinoid-treated maize seeds. Consequently, a new hypothesis was formulated, that bees obtain lethal doses by flying through the dust cloud created during planting activities. The reasoning behind this hypothesis is debatable. The authors state that exposure of bees from residues on vegetation surrounding drilled areas cannot be the key route of exposure because residue levels of neonicotinoids found in such vegetation is comparably low. Other authors (e.g. PISTORIUS et al. (2009), however, describe from the incident in the Upper Rhine Valley in Germany symptoms that clearly hint to effects caused by contaminated nectar or

pollen that was stored in the affected hives. Therefore it appears questionable in how far the initial hypothesis of the authors was correct.

In order to test the hypothesis that the acquisition of dust in flight is a significant source of bee contamination, the authors designed and conducted new experiments. TAPPARO et al. (2012) present planter emission data and individual bee exposure levels, however other papers present more clearly the toxic responses of honey bees to these exposures (e.g., see MAZARO et al. 2009 and GIROLAMI et al. 2012). Upon reading these other studies, it becomes clear that bee deaths generally did not occur unless the “powdered bees” were captured, brought into the laboratory and kept in conditions of high (>95%) humidity. As stated by MAZARO et al. (2009): “A clear indication that bees were killed by powdering, only if held in high humidity, emerged.” They did observe some individual bees killed in the field, but the level of mortality under typical field conditions was low. Air humidity values of > 95% are, at least under climatic conditions as prevailing in maize growing regions of the temperate zones, unusual; likewise, they are higher than humidities commonly prevailing in bee hives (see e.g. ELLIS 2008).

The experiments of this research team demonstrate that abraded dust from neonicotinoid-treated maize seeds is intrinsically very toxic to honey bees, and bee deaths can occur under artificial scenarios set up by the investigators. However, under normal agricultural and apicultural conditions, the sowing of neonicotinoid treated seeds did not pose a serious risk.

That abraded dust from insecticide-treated maize seeds is highly toxic to honey bees was already well known. Use of advanced seed coatings have been shown to significantly reduce abrasion of the a.i. from maize seeds. Modification of planter exhaust systems to direct airflow downward toward the soil has been shown to be very effective at reducing off-site drift. Ongoing product stewardship efforts continue to limit this and other routes of potential exposure to bees. The occurrence of bee poisoning events are now rare, and it is important to remember that even when such events have occurred, they have generally involved a small proportion of the bees in a colony and had no long-lasting effect. Even in the case of the 2008 incident in southern Germany in which an extraordinarily high level of seed dust was released due to improper coating of the treated seeds and over 12,000 bee colonies were affected, nearly all of these colonies recovered (PISTORIUS et al. 2009). There is no scientific justification for suggesting, as do TAPPARO et al. (2012), that abraded dust released during planting of neonicotinoid-treated maize seeds represents a possible cause of declining bee populations or colony collapse disorder, is of concern for crop production based on bee pollination, or is a widespread ecological problem requiring a rethinking of agricultural policies. The work of this research team demonstrates that lethal exposure of honey bees to abraded seed dust is only likely to occur under very specific and unusual conditions, and provides a basis for further targeted efforts to limit the possibility of such occurrences. A more reasoned interpretation of this and other recent papers on the same subject (e.g., KRUPKE et al., 2012) is that the problem of toxic exposure of bees to corn seed dust is limited in scope, and likely correctable with improved seed coatings/lubricants and planter modifications.

### **Correction of Technical Errors or Misleading Statements about Risk:**

Several statements made in the paper are incorrect or highly misleading.

In the Introduction section it is stated:

*“... the environmental releases of substances with recognized toxic and ecotoxic effects, such as neonicotinoid insecticides that have been associated with the worldwide crisis of honeybee colonies”*

Several studies have investigated whether pesticides, amongst them neonicotinoids, are a significant causative factor behind the decline of honeybee colonies. In the EFSA Scientific Report “Bee Mortality and Bee Surveillance in Europe” (HENDRIKX et al. 2009) it is pointed out that no involvement of pesticides has been proven for colony losses or CCD, and that “High concentrations of pesticides have rarely been identified in relation to colony losses in USA and Europe”. In a survey of the French AFSSA (2009b), pesticides were not among the key factors that were identified to be responsible for bee mortality; there was no evidence found for a significant involvement of pesticides in the observed bee declines. In an article published in “Science”, RATNIEKS & CARRECK (2010) point out that pests and diseases are the key factors behind bee decline, and that pesticides are unlikely to have caused bee losses as they were observed in the 1990s in France. These are just three examples of many examples that demonstrate that the scientific community does, with a few exceptions, not associate honeybee colony decline with pesticides.

In the Introduction section, it is further stated:

*“In the past decade honeybee colonies throughout the world have been subject to rapid losses in the order of 40%, in particular in Southern Europe.”*

According to POTTS et al. (2010), the number of honeybee colonies in Mediterranean Europe has increased by 13.3% during the last years. On a global level, numbers of colonies are likewise growing rather than decreasing according to AIZEN & HARDER (2009). Therefore the figure of 40% losses may refer to episodic events in certain countries or regions, but does not reflect a global trend nor even a regional trend for Southern Europe.

In the Introduction section, TAPPARO et al. (2012) go on to state:

*“This phenomenon, also named colony collapse disorder, represents a worldwide crisis with effects both on crop production and on ecosystems.”*

Colony Collapse Disorder is a phenomenon characterized by rather specific symptoms which is more or less restricted to USA; bee decline or mortality reported from other countries and regions is usually not associated with CCD-typical symptoms and is so far fundamentally different (see for instance HENDRIKX et

al. 2009, VAN ENGELSDORP et al. 2009, VAN ENGELSDORP & MEIXNER 2010). The authors are here mixing up different phenomena. Moreover, it is a myth that the observed bee decline is globally affecting agricultural crop production (e.g. AIZEN et al. 2008, AIZEN & HARDER 2009). There are no scientific data that substantiate the assumption that bee decline so far factually affects agriculture.

In the Introduction section, TAPPARO et al. (2012) state:

*“In Italy and Europe, corn sowing – from mid-March to May – was often accompanied by a rapid disappearance of foraging bees. [...] a close relationship was observed between the deaths of bees and the use of pneumatic drilling machines.”*

Apart from Italy, bee losses associated with maize drilling were only observed in Austria at a very low level, and in Germany at one and in Slovenia at two episodic events. From other European countries such observations have according to our knowledge never been made (see BARNETT et al. 2007, SEEFELD 2006, 2008, THOMPSON & THORBAHN 2009). In France, no cases of mortality caused by drilling of maize seeds treated with neonicotinoids have been found during extensive multi-year monitoring efforts (AFSSA 2007, 2009a).

In the Results and Discussion section, it is stated:

*“Germany –before the ban of neonicotinoids- and Austrian and Slovenian beekeepers continued to report extended losses of bee colonies in spring in conjunction with corn sowing.”*

In Germany and Slovenia, bee mortality associated with maize drilling was only reported in one or two, respectively, individual incidents. In Austria, only very few cases of increased mortality were reported. Colony losses associated with maize drilling have only very rarely been reported even in the scopes of the few incidents referred to in these countries (PISTORIUS et al. 2009, GIRSCH & MOOSBECKHOFFER 2011, MAROLT et al. 2011, SEEFELD 2006, 2008, THOMPSON & THORBAHN 2009).

In the section on Analytical Methods for Single-Bee Analysis after Field Exposure, it is stated:

*“Short-term mortality and the characteristic symptoms of neonicotinoid neurotoxicity [...]”*

Symptoms seen in bees that had been killed by neonicotinoids are not specific to this class of compounds (or not even specific to pesticide intoxication at all), so there is no way to diagnose neonicotinoid intoxication by means of externally visible symptoms found in dead bees.

## **Conclusion**

TAPPARO et al. (2012) present results of field experiments that demonstrate that honey bees that fly through the air exhaust of pneumatic corn planters can become contaminated with abraded dust from insecticide-treated maize seeds and this can sometimes result in the death of individual bees. However,

their research results and the available records of field incidents suggest that the problem of toxic exposure of bees to corn seed dust is limited in scope, and continues to be minimized with improved seed coatings/lubricants, planter modifications and product stewardship measures. This phenomenon has not been scientifically linked to, and is not suspected by mainstream scientists to be the cause of colony collapse disorder or widespread honey bee colony losses.

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Dr. David L FISCHER, Dr. Andrew CHAPPLE, Dr. Iain KELLY, Dr. Lubos VRBKA & Dr. Christian MAUS  
2012-03-25

## **Statement on the findings of the study:**

**LU et al. (2012):** In situ replication of honey bee colony collapse disorder

**Authors:** Ch. LU, K.M. WARCHOL & R.A. CALLAHAN

Harvard School of Public Health, Boston/USA

Worcester County Beekeepers Association, Massachusetts/USA

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### **Contents of the publication**

In their publication “In situ replication of honey bee colony collapse disorder”, LU et al. (2012) describe a trial in which they claim they have replicated inducing symptoms of Colony Collapse Disorder (CCD) by chronically exposing bee colonies to high-fructose corn syrup (HFCS) contaminated with Imidacloprid, and bring forward a new hypothesis about the origin of CCD. The authors hypothesize that CCD is caused by Imidacloprid residues originating from seed treatment in corn in HFCS which is used to feed honeybee colonies. In order to verify this hypothesis, they set up the following trial design: In each of four different apiaries, five commercial colonies were set up, four were fed with HFCS containing Imidacloprid at different concentrations, the fifth received uncontaminated HFCS and served as control colony. The colonies of the treatment groups were first exposed to a lower Imidacloprid concentration for 4 weeks, followed by a higher exposure concentration for subsequent 9 weeks. The exposure concentrations of the different treatment groups were 0.1 + 20 µg/kg, 1.1 + 40 µg/kg, 5.3 + 200 µg/kg, and 10.5 + 400 µg/kg. Treatments were repeated weekly between July and September 2010. In this period, hives were assessed weekly, with bi-weekly brood assessments. Further weekly hive assessments followed in the time period between December 2010 and March 2011. At the end of the assessment period all colonies of the two higher treatment group and the 20 µg/kg treatment group, three out of four colonies of the 40 µg/kg group, and two out of four colonies of the control group were found dead. From this observation, the authors conclude that the observed mortality, which they attribute to exposure to Imidacloprid, demonstrate that Imidacloprid residues in HFCS are a plausible mechanism to explain CCD.

### **Comments from the BCS perspective**

It is obvious that the study design applied by LU et al. (2012) suffers from many flaws, with regard to the set-up of the trial as well as regarding the context in which it was conducted. Moreover, many of the conclusions drawn from the findings are highly questionable. In the following, the most critical points are listed and discussed.

**Trial Design – Replication.** In ecotoxicological bee testing, especially when entire honeybee colonies are subject of the test, the choice of a suitable design is of key importance for obtaining scientifically solid results. An important aspect of this is an appropriate replication, especially since a bee colony is a highly complex meta-organism, a fact which entails a significant natural variability in almost all testing endpoints that may be measured in such a study. The numbers of replicates that are needed to appropriately address a certain endpoint depend largely on the complexity and intrinsic variability of the endpoint to be measured. For a relatively simple endpoint like mortality or foraging activity, relatively low replicate numbers like three or four, as normally used in semi-field tests, are commonly considered appropriate. However, this cannot be applied to the study under discussion here. One of the most complex endpoints one could think of is overwintering success and overwintering mortality of bee colonies – an endpoint that depicts the upshot of a long, complex process that is influenced by numerous, partly interdependent variables and factors, many of which are not even known to us. To address this kind of endpoint with a relatively low number of replicates, as Lu et al. (2012) did, (only four hives per treatment group!) seems hardly appropriate. Therefore it is questionable in how far their design is at all capable of detecting significant differences between different experimental groups in the endpoint overwintering mortality. The authors seem to be aware of this as they call this weakness of their study design an “apparent deficiency”. It remains the question then: why do they nevertheless draw conclusions from a test conducted under an obviously flawed design? In this context, it is likewise notable that the authors apparently did not conduct a statistical analysis of their colony mortality data, as would be expected in a solid scientific study.

In addition to the low numbers of colonies of the individual treatment groups that actually do not allow drawing conclusions from the outcome of the study, similarly the dose-response relationship in colony mortality that the authors claim to see, is rather doubtful: in the 20 µg/kg exposure group, mortality set in earlier than in the 40 µg/kg as well as in the 200 µg/kg treatment group, and reached higher levels than in the 40 µg/kg group. This is not consistent with the assumption of a dose-response relationship. Likewise, the authors state that they have “found that the initial brood rearing corresponded to imidacloprid doses two weeks after the initial imidacloprid dosing, however, it is inversely related to imidacloprid dosages at the end of dosing regime”, however, they then admit that the “decrease [of brood cells during the observation period] is independent of different Imidacloprid doses applied to the hives“. According to the graph shown in Figure 1, there is no obvious or significant dose-response relationship in the abundance of brood cells at all – values fluctuate, as typical for this endpoint, over time, with sometimes the control group, sometimes a treatment group (in particular the 20 µg/kg and the 40 µg/kg groups) having highest abundances of brood cells. Towards the end of the season, brood abundance decreases in all treatment groups, which is a natural phenomenon related to the generation cycle of a bee colony. During this period, measurements of brood abundance will anyway lead to erratic figures, so there is no sense in making comparisons between observations on the development of this endpoint in different treatment groups during this time of the year.

Another point that raises some doubt that the test design as used by Lu et al. (2012) would be capable of reliably detecting treatment effects and yielding anything else but erratic results, is the following: there is no doubt that dietary Imidacloprid concentrations of 400 or even of 200 µg/kg are hazardous to bees, especially when chronically administered to a colony. When a colony is exposed to such concentrations over many weeks, one should expect that there would be effects of this visible. In so far it is surprising that apparently no such effects were seen at all during all the exposure period, and that first signs of “treatment-related” colony mortality only set in 13 weeks after exposure. This suggests that the test design was not sufficiently sensitive to detect any treatment-related effects, and the distribution of the observed mortality over the different treatment groups was just erratic.

**Exposure of Bee Colonies to Neonicotinoids.** In a sound scientific approach, it would have been the logical procedure to first gather information about the nature of a realistic exposure scenario, before simulating this scenario in a trial. In the given case, this would have meant evaluating what residue levels of Imidacloprid would normally be present in HFCS, and then defining the exposure levels to be tested in a study on the basis of these realistic exposure levels. However, apparently the authors had no information available about potential residue levels in HFCS, and instead of determining a realistic exposure scenario by conducting residue analyses in commercially available HFCS samples, they based the exposure levels tested in their study on pure guesses which are completely unsubstantiated. They argue that the maximum residue level of Imidacloprid in corn as set by US EPA is 50 µg/kg. As there is no maximum residue level set for HFCS, they arbitrarily assume that residue levels in HFCS are the ten-fold maximum tolerated level for corn grain, from which HFCS is processed. First, deducing an anticipated, common environmental exposure concentration from a maximum tolerated residue level is absurd and not in compliance with any scientifically sound principle. Second, a ten-fold extrapolation factor for a product which is even not processed in a concentration process (see below) is by no means justified (analogy: this would be like assuming that each pedestrian walking on the sidewalk along a street with a 100 km/h speed limit would move there with a speed of 1000 km/h – the speed limit is basically valid for cars, so other entities may move at much higher speeds, even though common sense suggests that they would not do so).

Another point which is very questionable related to the author’s assumption that there were significant Imidacloprid residues in HFCS refers to the following consideration: if their assumption was true that there is widespread occurrence of residue levels of Neonicotinoids in HFCS, why did they then not analyze the HFCS they used for Neonicotinoid residue levels just to determine these levels, and why did they then have to spike it with Imidacloprid at all – if their hypothesis was correct, then residues of the compound would already have been in there, and spiking it again would have been double dosing. Then, if there would be significant residue levels of Imidacloprid in commercially available HFCS, why did the authors then find no residues in their analysis of the blank sample fed to their control hives (Table 2)? Implicitly, the authors seem to suggest that a problem with Imidacloprid residues in HFCS must have been in

particular in older batches produced in 2005/06, as they point out that they were not able to obtain HFCS manufactured during this years, so they “used food-grade HFCS fortified with different levels of Imidacloprid, mimicking the levels that are assumed to have been present in the older HFCS.” This approach is lacking any logical coherence – if a significant residue level of Imidacloprid was present in HFCS in 2005/06, but no more nowadays, how can the incidences of CCD symptoms that are still reported in more recent years, be attributed to Imidacloprid residues in HFCS? Moreover, the authors give no explanation why in the framework of their hypothesis Imidacloprid residues in HFCS should have been different between 2005/06 and nowadays.

Then, even if there were Imidacloprid residues in HFCS as the authors hypothesize, the exposure scenario as tested would not be realistic: in apicultural practice, no bee colony would be fed with HFCS all season long. In so far, exposure conditions as applied here would be strongly exaggerated in any case.

Whatever assumptions about residues of Neonicotinoids may be present in HFCS might have been made by the authors, it seems, in contrast to their hypotheses, to be a matter of fact that HFCS contains no Neonicotinoid residues at all. In 2009, USDA analyzed 12 HFCS samples of different origins for pesticide residues; these residue analyses screened for ca. 200 active ingredients; the detection limit for Imidacloprid was 1 µg/kg. No residues of Imidacloprid or other Neonicotinoids were found (Roger Simonds, USDA Agrimarketing Service, Gastonia, North Carolina, personal communication). Therefore, it seems very unlikely that residues of Imidacloprid or other Neonicotinoids are prevalent in HFCS.

That no Imidacloprid residues are found in HFCS that is produced from seed-treated corn is not surprising when considering the process in which this commodity is usually produced: Corn grain is converted to corn syrup with several production steps. First, the grain is wet milled to produce corn starch. The starch is taken through three separate enzymatic conversions to break the starch into oligosaccharides, then to hydrolyze the oligosaccharides to glucose, and finally to isomerize the glucose to a glucose/fructose mixture. The raw corn syrup is then purified with activated carbon. Even if Imidacloprid residues that might potentially be present in the grains would not be degraded in the maceration process, they would ultimately be removed from the syrup by final activated carbon purification. Moreover, there is no step in this production process in which Imidacloprid could be concentrated. Therefore, even if there would be residues in HFCS, they would be lower rather than higher than those that might be found in corn grain.

Another point to justify the tested concentrations that the authors are bringing forward is a reference to a paper of GIROLAMI et al. (2009) who found Imidacloprid residue levels of 47 mg/kg in guttation liquid from seedlings of Imidacloprid seed-treated maize plants. It is not in any way reasonable to compare residue levels in xylem liquid of young seedlings (this is where guttation fluid originates from) with residue levels in processed products originating from fruits from mature plants in which the substance used for seed treatment has been massively diluted and degraded. Moreover, it has been convincingly demonstrated that guttation liquid is not normally used by bee colonies as relevant water source (KEPPLER et al. 2010, PISTORIUS et al. in press), and even in cases where individual bees might be picking up guttation fluid, this would not lead to a long-term exposure of the colonies as it was in the design of the study of LU et al.

(2012). The authors state then that “the finding of the loss of honey bee hives at the levels as low as 20 µg/kg of imidacloprid in HFCS raises the question of whether there is a no observed- adverse-effect-level of imidacloprid (and most likely of other neonicotinoids as well) for honey bees.” This statement is erroneous: A NOAEC for Imidacloprid has been established in numerous honeybee studies under field-relevant conditions (see for instance MAUS et al. 2003, SCHMUCK et al. 2005). That the authors were not able to determine a NOAEC in their study may on one hand be related to the fact that the described experiment was probably not capable of establishing a robust NOEC for the endpoint overwintering mortality due to flaws of the design (see above). On the other hand, the authors tested, compared to environmental exposure levels, excessively high dietary concentrations: even the lowest exposure level tested (20 µg/kg) is by far higher than the residue levels that are normally found in nectar and pollen of seed-treated crops (see for instance MAUS et al. 2003, SCHMUCK et al. 2005), and the highest concentrations tested by LU et al. (2012) were hundred-fold and more overdosed compared to typical environmental concentrations.. The authors argue that the bees of the test colonies would have reduced the concentration of Imidacloprid in their food supply by diluting the offered HFCS with nectar collected from nearby floral resources, but to reach realistic exposure levels, even the lowest test concentration would have to be diluted by a factor of four to ten, which is, under consideration of the season when exposure took place (end of July to end of September when natural food sources for bees tend to be scarce), highly unlikely. A chronic dietary exposure to residue levels as high as 20 µg/kg, as tested in the study under discussion here is therefore a scenario that would not occur under realistic agricultural conditions. Interestingly, in an earlier study on overwintering success of bee colonies exposed to Imidacloprid (FAUCON et al. 2005), where environmentally more relevant residue levels (0.5 and 5 µg/kg) were tested with more appropriate replicate numbers (8 to 9 per treatment group), no adverse effects to the exposed hives were found.

Another debatable point regarding the dosing regimes applied in the study under discussion here is why the authors first applied low exposure concentrations between 0.1 and 10.5 µg/kg which, at least the lower concentrations, may come close to what a bee colony may be exposed to in the field, but then after a few weeks switched to at least partly dramatically exaggerated rates. There seems no be no reasonable rationale behind this, and the authors likewise do not justify this unusual dosing regime.

**Correlations between colony mortality and the use of Neonicotinoids.** The authors claim that the first significant occurrence of CCD in US in 2006/07 was correlated with the introduction of neonicotinoid insecticides as seed treatment in corn in 2004/05. This, again, is a statement which is based on several misconceptions and errors: 1.) Imidacloprid as a seed treatment in corn was brought on the market in US in 2000, and was first used there during the planting season 2001. 2.) Imidacloprid seed treatment in corn was initially only applied on a very limited acreage in US, and after two years on the market, it was hardly used any more, being replaced by other products. During the years 2004 to 2011, the percentage of US corn acres treated with Imidacloprid has been less than one-half of one percent (BCS, unpublished data).

Therefore it is ridiculous to assume that after market introduction of Imidacloprid in corn, Imidacloprid residues might have been prevalent in HFCS produced in US, and the claimed correlation is completely unsubstantiated.

If there would exist a causative link between the use of Neonicotinoids and honeybee colony mortality, one should see a correlation between use of Neonicotinoids and exposure of bees to Neonicotinoids on one hand, and colony losses on the other hand. However, this is not the case at all. On the contrary, CCD occurrence and other colony losses that have been observed at large scales are not correlated with exposure of honey bee colonies to Neonicotinoids (VAN ENGELSDORP et al. 2009, DELAPLANE 2012) or to exposure of colonies to Neonicotinoid-treated crops (e.g. OTTEN 2003a, b, CHARRIÈRE & NEUMANN 2010). Linkage of Neonicotinoid exposure to declining bee colony health and elevated colony losses has not been found in any of the recent regional multifactorial studies of declining bee health (VAN ENGELSDORP et al. 2009, 2010a, ROGERS & KEMP 2004, NGUYEN et al. 2009, CHAUZAT et al. 2009, GENERSCH et al. 2010). As another example, no unusual colony losses are reported from Australia, where a lot of Neonicotinoid seed treatment is applied (but no *Varroa* mites are present) (NEUMANN & CARRECK 2010). Moreover, in recent scientific reviews of the evidence for whether Neonicotinoid pesticides play a causal role in bee declines (BLACQUIERE et al. 2012, CRESSWELL et al. 2012), the conclusion reached is there is no evidence that they do. Finally, if increased colony mortality would in fact be caused by Neonicotinoid-contaminated HFCS, how could then the observed colony losses in regions where HFCS is or has not been not commonly used to feed bee colonies (like Europe, Asia) be explained?

**Symptoms of mortality.** The authors claim to have induced CCD in their study. However, the symptoms described do not seem to support this claim. In late December 2010, all colonies were found alive, but the ones exposed to higher levels of Imidacloprid “appeared” weaker with smaller bee clusters inside the hives (apparently just an estimate), and dead bees were found in front of the hives. Then subsequently, more and more colonies died after these signs of weakening. The dead hives were “remarkably empty”, just food stores with honey and pollen were left in the combs. VAN ENGELSDORP et al. (2009) describe in detail the symptoms used to define colonies as suffering from CCD. Symptoms include (1) “the apparent rapid loss of adult worker bees from affected colonies as evidenced by weak or dead colonies with excess brood populations relative to adult bee populations, (2) a noticeable lack of dead worker bees both within and surrounding the affected hives, (3) the delayed invasion of hives pests (e.g. small hive beetles and wax moths), and kleptoparasitism (stealing food) from neighboring honey bee colonies”. Other authors (summarized by HENDRIKX et al. 2009) add that CCD is furthermore characterized by a sudden disappearance of worker bees during the beekeeping season, and by evidence of recent brood (young larvae, seemingly healthy queen). Almost none of these symptoms was observed in connection with the colony mortality described by LU et al. (2012): the disappearance and the collapse of the affected colonies did apparently not set in spontaneously, but was preceded by a weakening of the colonies; moreover, dead bees were found in front of the hive, which would by definition not be the case in incidences of CCD,

but which is normal for overwintering colonies. There is no mention of the presence of a surviving queen in the affected hives, but it can be implied that the authors would have recorded the presence of a lonesome queen in the “remarkably empty” hives. Moreover, there is no information provided about the presence of bee brood, but since the observation were made during winter time, it can be assumed that there was no brood. Thus, what the authors have seen was colony mortality, but certainly not CCD.

**Potential causes of mortality.** The authors claim that “The loss of imidacloprid-treated hives in this study is also highly unlikely due to pathogen infection since the presence of neither *Nosema* nor a large number of *Varroa* mites was observed in hives during the summer and fall seasons.”, and they conclude that “Since all hives were considered healthy as they went into fall season, those pathogens posed very little threat to the health of honey bee hives.” They substantiate this by an Apistan® (active ingredient: Tau-Fluvalinate) and Fumagillin B treatment against *Varroa* and *Nosema* that had been conducted in October. Apparently, however, the health status of the colonies and the presence or absence of parasites was not accurately checked with appropriate methods (or, if so, the results of this health analysis, like *Varroa* counts or *Nosema* spore counts, are not documented), and it appears that hives were simply declared healthy by the authors based on the lack of obvious signs of disease or parasite infestation seen by visual inspection. However, the absence of conspicuous signs of diseases and parasites in late summer is by no means conclusive evidence that the hives were not infested at all. Many beekeepers who have lost their hives over winter due to Varroosis or other diseases would have considered them healthy when visually inspecting them in late summer or fall. Likewise, just the fact that a treatment against a certain parasite has been conducted does unfortunately by no means guarantee that the treatment was successful and that the treated hive was and has remained parasite-free later on, especially when the treatment was conducted with an inappropriate timing (see below), and with an active ingredient that might be not efficient, if possible resistance has not been checked. For instance, according to OTTEN (2005), German apiaries which had been treated with Apistan® had losses as high as 43% over the winter of 2002/03. As the authors likewise do not provide any data about diagnosis of diseases or parasites in the colonies that died over the winter, it is very possible that the observed mortality was caused by *Varroa* or other parasites or diseases which are commonly recognized to be the main factor behind colony overwintering mortality.

**Hive management.** In the publication of LU et al. (2012) there are some details described about the handling of the study colonies that give rise to the assumption that they have been improperly managed. For instance, *Varroa* treatment with Apistan® was conducted in October, which is much too late in the season to be fully effective. Moreover, the applied product is not effective against mites in many areas of the US. It is very possible that these management practices have contributed to the overall high colony mortality observed in this study. Another point to mention is that the hives were apparently opened once a week for assessments, even during the winter months at low temperatures. This is certainly not beneficial

for colony health and may likewise have weakened them. In this context it is notable that hive management is in fact an important factor causing colony losses – VAN ENGELSDORP et al. (2010b) even rank it higher than CCD as a factor causing colony mortality.

**Errors.** Finally, there are several statements in the paper that are simply untrue or incorrect in the given context. These include:

*“The abrupt emergence of colony collapse disorder (CCD) in the United States during 2006-2007, and other countries later has raised the concern of losing this important perennial pollinator globally.”* - Large-scale losses are not new to the beekeeping industry. Many of the symptoms similar to those related to CCD have been described before. The first published record of this disorder appeared already in 1869. Subsequently losses were described in Colorado in 1891 and 1896 where large clusters disappeared or dwindled to tiny clusters with queens in May, hence the name “May disease” (UNDERWOOD & VAN ENGELSDORP 2007). Then, CCD is not a common, global phenomenon, but specific to USA; in Europe, for instance, colony losses with CCD symptoms are exceptional (see for instance HENDRIKX et al. 2009, VAN ENGELSDORP et al. 2009, VAN ENGELSDORP & MEIXNER 2010). Even in the USA, many professional apiarists have never seen a single case of CCD. The vast majority of colony losses in the US are not from CCD (see e.g. VAN ENGELSDORP et al. 2010b).

*“Although some losses of honey bees from healthy and well managed hives during the winter months have always been part of apiculture (for instance, in the New England area, winter losses of honey bee hives are typically 15-30%), never in the history of the beekeeping industry has the loss of honey bee hives occurred in such magnitude and over such a widely distributed geographic area.”* – Sadly enough, beekeepers are not facing only “some losses” and certainly not limited to the New England area. According to NEUMANN & CARRECK (2010) elevated colony losses have recently been reported from e.g. Europe (CRAILSHEIM et al., 2009), the USA (VAN ENGELSDORP et al., 2009; 2010) and the Middle East (HADDAD et al., 2009, SOROKER et al., 2009) but only rarely from South America and virtually not at all from Africa and Australia. The scientists cited above linked this to the fact that colonies of African honey bees and Africanized honey bees in South America survive without *Varroa* treatment, whilst the mite has not yet introduced into Australia. Furthermore, bee colony losses are clearly not a new phenomenon, they just gained more attention: VAN ENGELSDORP & MEIXNER (2010) examined the historical records and showed that extensive losses are not unusual. Already almost a century ago, in 1906, beekeepers on the Isle of Wight (England), noticed that many of their honey bee colonies were dying, with numerous bees crawling from the hive unable to fly. Older records report massive bee colony losses without obvious disease symptoms from Australia in 1872 (BEUHNE 1910).

*“Winter losses of honey bee hives usually occur because honey bees run out of or cannot access food, or the cluster becomes too small to generate sufficient heat.”* – Starvation is one of the potential causes of overwintering mortality, however, clearly not the only or even not the most predominant one. Factors involved in overwintering mortality are for instance listed by VAN ENGELSDORP et al. (2010b) and by BRODSCHNEIDER et al. (2010).

*“Commercial beekeepers appear to be affected by CCD at a disproportional rate”* – According to van ENGELSDORP et al. (2010b), professional beekeepers record on average less pronounced losses compared to small apiaries.

To explain that mortality only set in several months after exposure to the treatment, the authors hypothesize that winter bees were in their larval stage exposed to Imidacloprid, and therefore may have been sublethally affected by this as adults. As an evidence for this kind of phenomenon, they cite the publication of MEDRZYCKI et al. (2010). However, this paper describes different sensitivities of bees that were reared at different temperatures to an insecticide, and not a reduced vitality of bees that were reared exposed to a pesticide. In so far, this study is not of relevance in the given context.

## **Conclusions**

The study presented by Lu et al. (2012) has been conducted according to a faulty design that was based on numerous incorrect to unsupportable assumptions which are totally inconsistent with a sound scientific approach. The results are accordingly insignificant for any risk assessment, moreover the authors interpret them in a very questionable way. The study provides no evidence at all that the exposure of honeybee colonies to Neonicotinoids under realistic conditions might have any adverse effect.

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